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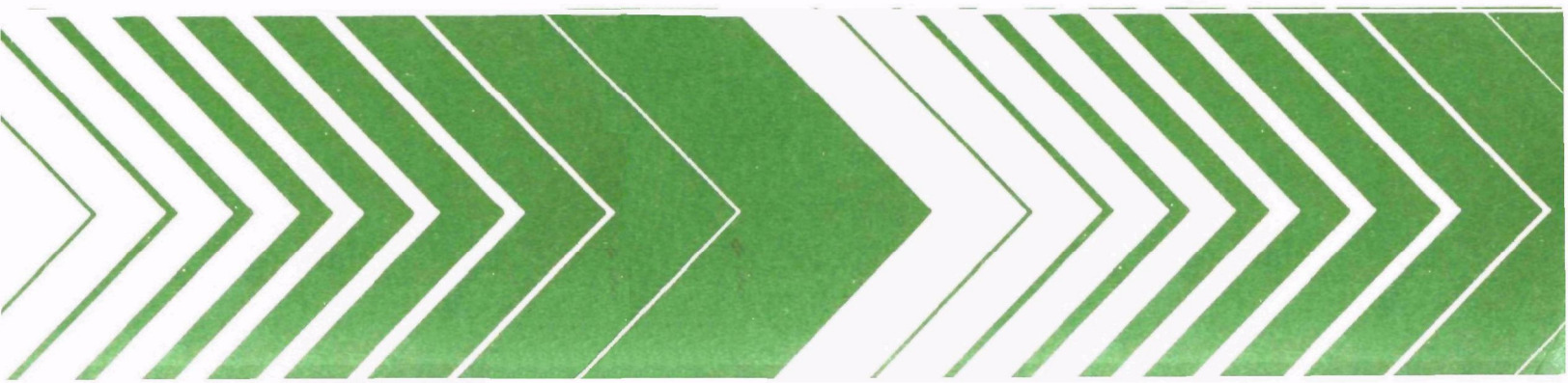
Selected Toxicological Studies of Dimilin in Weanling Male Rats



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SELECTED TOXICOLOGICAL STUDIES OF DIMILIN IN WEANLING MALE RATS

by

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FOREWORD

Protection of the environment requires effective regulatory actions based on sound technical and scientific data. The data must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of exposure to specific pollutants in the environment requires a total systems approach that transcends the media of air, water, and land. The Environmental Monitoring Systems Laboratory at Las Vegas contributes to the formation and enhancement of a sound monitoring-data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report provides significant new data on the toxicological response of weanling male rats to the pesticide, Dimilin. This study specifically deals with the effects of Dimilin on the circulating testosterone and the development of the reproductive organs. For further information, the reader should contact the Exposure Assessment Division.

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ABSTRACT

Effects of subacute doses of Dimilin [1(4-chlorophenyl)-3-(2,6-difluorobenzyl)urea] on the reproductive system of weanling male rats were examined over a period of 96 days. The parameters evaluated were: testosterone level in plasma, growth of reproductive organs (testes, prostate, seminal vesicles) and adrenal glands, and histological examination of tissues for pathological changes associated with the administration of Dimilin. By intragastric intubation, groups of male rats, 25 days old, were given 0, 15, 150, or 300 milligrams of Dimilin suspended in vegetable oil per kilogram per day for a period of 0, 14, 28, 42, and 96 days.

The data indicate that Dimilin had no adverse effects on body weight or organ weights of weanling rats, but decreased the levels of testosterone in the plasma of animals of prepubertal age. However, this effect of Dimilin began to disappear with the onset of puberty. Histological examination of the test animals having lower levels of testosterone in plasma failed to reveal any Dimilin-induced changes in interstitial or germinal cells. On the basis of these observations, it is concluded that Dimilin, at dose levels of 15, 150, and 300 milligrams per kilogram per day, transiently depresses the testosterone in plasma during the prepubertal period, yet has no delaying effects on the development of the reproductive organs of male rats.

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INTRODUCTION

Insect growth regulators are a new group of compounds that disrupt growth and normal development of the insect in various immature stages (1). One of these compounds, the insecticide Dimilin (Diflubenzuron; TH 6040; 1(4-chlorophenyl)-3-(2,6-difluorobenzyl)urea), has emerged as one of the promising urea-based larvicides. This compound prevents the incorporation of glucose into endocuticle through the inhibition of chitin synthesis (2). Dimilin has been successfully used in controlling a variety of arthropod pests injurious to plants and animals (3,4). This compound, when spread directly onto cattle, effectively reduces the egg hatching of stable flies and horn flies. Secretion of Dimilin into the egg damages the developing embryo which accounts for the "sterility" observed in these insects. This compound has also shown excellent potential for controlling the larval stages of mosquitos (5,6), Diptera (7,8), certain Lepidoptera (9), and Coleoptera (10).

Dimilin's biotransformation in lactating cows, castrated sheep, and rats has been reported. It appears to be absorbed, metabolized, and excreted in the urine. The major metabolites of Dimilin excreted by the cow, sheep, and rats are the result of the hydroxylation of difluorobenzyol and chlorophenyl rings and the cleavage between the carbonyl and amide groups. The mutagenic potential of the diflubenzuron metabolites has also been investigated using the Salmonella mutagenicity test (11).

On account of Dimilin's high toxicity toward many destructive insects and its extremely low toxicity in rats (Oral LD_{50} >10 g/kg) and mice (LD_{50} >4.64 g/kg), Dimilin has been proposed for use as an insecticide on commercial crops for control of such pests as cornborers. However, it has been reported that when Dimilin was given in feed to baby chicks for 13 weeks, the males failed to mature--the combs, wattles, feathers, and voice remained undeveloped. These adverse effects with Dimilin were accompanied by decreases in plasma testosterone proportional to Dimilin dosage (12).

Thus, in view of these adverse findings, and the lack of information concerning other possible effects of Dimilin on the reproductive system of weanling male rats (in terms of plasma testosterone, development of reproductive organs), the present study was undertaken.

CONCLUSION

When weanling male rats were given daily doses of 15, 150, and 300 mg/kg body weight of Dimilin suspended in oil over a period of 14, 28, 42, and 96 days, Dimilin produced a transient depression in plasma levels of

testosterone in the prepubertal period without causing any noticeable delaying effect on the development of the reproductive organs.

EXPERIMENTAL PROCEDURES

ANIMALS AND DOSAGE

Male Long-Evans rats were obtained as weanlings from Blue Spruce Farms, Inc., Altamont, New York 12009. They were acclimated to a controlled animal room environment with a light cycle of 14 hours of light per 10 hours of darkness for a period of 4 days. The rats were approximately 25 days old (55 g mean body wt) at the initiation of the study. Dimilin (U.S. EPA Lot no. PP312; 99% purity) was administered daily by intragastric intubation as a suspension in vegetable oil to groups of rats throughout the duration of the experiment. The control animals were given only vegetable oil. The dosage levels of Dimilin were 0, 15, 150, and 300 milligrams of Dimilin per kilogram of body weight.

EXPERIMENTAL DESIGN AND CONDUCT

On the day before the start of pesticide administration, weanling male rats were weighed and randomly assigned to control and treatment groups. Those rats in the lower and upper ranges of body weights were first eliminated. The control group contained 15 animals and each test group consisted of 8 animals to provide optimal observations and contrast for statistical evaluation. The experimental design is presented in Table 1.

TABLE 1. EXPERIMENTAL DESIGN

Group	No. of Controls (oil only)	No. of Low Dose Rats	No. of Medium Dose Rats	No. of High Dose Rats	Day of Sampling and/or Sacrifice
I	15*	-	-	-	1
II	15	8	8	8	14
III	15	8	8	8	28
IV	15	8	8	8	42
V	15	8	8	8	96

* Controls for Group I received no treatment; controls for other groups received vegetable oil, without Dimilin, by intragastric intubation.

All animals were housed individually in galvanized metal cages with food and water supplied ad lib. Body weights were recorded weekly and the dosage was adjusted according to the mean body weight to attain the specified dosage levels.

On the day treatment began, blood samples from 15 non-treated animals (Group I, Table 1) were collected for testosterone analysis. The animals were sacrificed; organ weights of testes, prostate, seminal vesicle, and adrenal glands were obtained and the tissues preserved in 10 percent neutral buffered formalin. The remaining groups of animals were sacrificed at the same time of day throughout the experiment to minimize variation due to diurnal rhythms of circulating testosterone. Handling, ambient environment, food, water, etc., were consistent for all animals. Wet weights of the above mentioned organs were recorded and the organs were fixed in 10 percent neutral buffered formalin.

The testosterone concentrations in plasma for the control animals and the test groups were determined by a radioimmunoassay procedure and the analyses were carried out by Smith Kline Clinical Laboratories, Inc., Burbank, California. Testes from all groups (control and treatment groups) and the adrenal glands from animals of Group III (Table 1) were submittted for histopathological evaluation.

All histology and pathology examinations were performed on 5-micron sections stained with hemotoxylin and eosin by Dr. B. D. Ward, Veterinary Pathologist, Mississippi State University, Starkville, Mississippi.

The statistical analysis was performed on arithmetic means from log-transformed data and employed analysis of variance procedures.

RESULTS

The mean body weights of the control and treatment groups following daily administration of Dimilin are given in Table 2. The results show that after 14, 28, 42, and 96 days of Dimilin treatment there are no significant differences in body weights between the control and Dimilin-treated rats.

Organ weights for the various treatment groups are given in Table 3. One-way analysis of variance did not reveal any dose-related changes in the various organ weights among the different groups. There are some minor differences observed, however. The only consistent observation is a significant decrease in the relative adrenal gland weights of all animals on Dimilin for 28 days.

The values for the plasma testosterone levels are given in Table 4. The animals in Group II, which had received Dimilin for 14 days revealed a decrease in plasma testosterone at all three dosage levels. Lower levels of testosterone were also observed in animals receiving Dimilin for 28 days at

TABLE 2. EFFECT OF DIMILIN ON BODY WEIGHT OF MALE RATS FOLLOWING DAILY ADMINISTRATION OF DIMILIN

	Weights (g) are expressed as mean ± S.D.				
Group	Control[a]	15 mg/kg/day[b]	150 mg/kg/day[b]	300 mg/kg/day[b]	Remarks
I	54.3 ± 4.7	-	-	-	25 days of age
II	123.8 ± 10.5	124.3 ± 10.4	125.5 ± 8.7	122.8 ± 14.5	14 days on Dimilin 39 days of age
III	212.3 ± 19.1	214.1 ± 23.6	221.5 ± 16.5	209.0 ± 14.4	28 days on Dimilin 53 days of age
IV	298.1 ± 35.2	291.8 ± 26.5	307.3 ± 25.9	301.1 ± 33.3	42 days on Dimilin 67 days of age
V	481.3 ± 34.3	488.6 ± 28.5	479.6 ± 21.0	465.1 ± 35.2	96 days on Dimilin 121 days of age

[a] = 15 animals each
[b] = 8 animals each

150 mg/kg/day, and at 300 mg/kg/day, and in animals receiving Dimilin for 42 days at 150 mg/kg/day. However, it is interesting to note that by day 96, the levels of plasma testosterone in the treated animals had increased. At day 96, the plasma testosterone values of Dimilin treated animals were not significantly different from the plasma testosterone values of the controls.

The histological evaluation of the testicular tissues of the control and treated groups and the adrenal glands of Group III (Table 1) animals, revealed no histopathological changes attributable to Dimilin.

Eighteen plasma samples (12 experimental plasma samples and 6 samples from pooled plasma of untreated animals) were submitted for an inter-laboratory cross-check comparison to Endocrine Sciences, Tarzana, California. The results of the entire 18 samples were subjected to the t-test for

TABLE 3. EFFECT OF DIMILIN ON ORGAN WEIGHTS OF MALE RATS FOLLOWING DAILY ADMINISTRATION OF DIMILIN

Group	Organ Weights (mg) are expressed as mean ± S.D.						
			Seminal Vesicles				
	Testes	Prostate	With Fluid	Intact	Without Fluid	Adrenal	Remarks
I							
Control[a]	337.3 ± 42.3	11.7 ± 4.3	-	23.6 ± 7.0	-	21.0 ± 3.5	25 days of age
II							
Control[a]	1156.3 ± 257.5	43.3 ± 9.2	-	40.3 ± 7.4	-	40.5 ± 8.4	14 days on Dimilin
15 mg/kg/day[b]	1209.0 ± 150.2	47.9 ± 7.1	-	45.3 ± 11.7	-	35.6 ± 5.7	39 days of age
150 mg/kg/day[b]	1252.0 ± 118.4	48.0 ± 6.0	-	41.9 ± 8.5	-	37.8 ± 2.4	
300 mg/kg/day[b]	1178.8 ± 211.9	44.6 ± 14.1	-	44.6 ± 8.3	-	41.5 ± 7.6	
III							
Control[a]	2341.4 ± 197.0	116.8 ± 34.9	-	210.1 ± 54.4	-	69.9 ± 11.6	28 days on Dimilin
15 mg/kg/day[b]	2246.3 ± 298.4	94.0 ± 26.5	-	193.3 ± 49.4	-	60.8* ± 7.8	53 days of age
150 mg/kg/day[b]	2283.0 ± 270.6	102.4 ± 16.4	-	196.6 ± 38.4	-	59.8* ± 4.8	
300 mg/kg/day[b]	2277.5 ± 308.4	103.4 ± 11.6	-	211.3 ± 61.0	-	60.3* ± 3.8	
IV							
Control[a]	2965.5 ± 361.5	165.1 ± 51.2	661.0 ± 187.7	-	267.3 ± 57.5	53.3 ± 10.8	42 days on Dimilin
15 mg/kg/day[b]	2931.6 ± 239.9	155.6 ± 35.4	607.6 ± 53.5	-	249.3 ± 28.3	47.4 ± 9.9	67 days of age
150 mg/kg/day[b]	2915.3 ± 204.3	176.1 ± 64.8	699.3 ± 169.6	-	287.8 ± 75.4	47.9 ± 4.3	
300 mg/kg/day[b]	2918.8 ± 257.2	163.5 ± 52.4	632.8 ± 154.5	-	239.4 ± 51.7	46.1 ± 4.2	
V							
Control[a]	3448.1 ± 239.5	345.7 ± 33.8	1244.5 ± 187.5	-	360.3 ± 46.4	58.3 ± 13.0	
15 mg/kg/day[b]	3451.5 ± 131.1	356.4 ± 43.5	1264.1 ± 186.6	-	358.3 ± 77.9	61.8 ± 15.3	96 days on Dimilin
150 mg/kg/day[b]	3510.6 ± 232.9	344.1 ± 59.5	1259.9 ± 158.1	-	353.3 ± 41.1	58.6 ± 10.4	121 days of age
300 mg/kg/day[b]	3644.5* ± 200.1	347.6 ± 64.6	1336.5 ± 116.8	-	369.9 ± 46.9	56.3 ± 8.8	

[a] = 15 animals each
[b] = 8 animals each
* Significantly different from Control P<.05 (one-way analysis of variance)

TABLE 4. EFFECT OF DIMILIN ON PLASMA TESTOSTERONE LEVEL IN MALE RATS FOLLOWING DAILY ADMINISTRATION OF DIMILIN

	Plasma Testosterone Values are expressed as Mean ± S.D. (ng/l))				
Group	Control[a]	15 mg/kg/day[b]	150 mg/kg/day[b]	300 mg/kg/day[b]	Remarks
I	13.3 ± 8.6	-	-	-	25 days of age
II	74.7 ± 81.3	21.4 ± 57.2*	16.7 ± 34.2*	4.2 ± 4.3*	14 days on Dimilin 39 days of age
III	213.3 ± 163.4	217.8 ± 96.9	115.5 ± 77.5*	113.6 ± 72.3*	28 days on Dimilin 53 days of age
IV	480.0 ± 264.2	319.8 ± 328.5	145.0 ± 120.2*	353.3 ± 200.2	42 days on Dimilin 67 days of age
V	177.9 ± 112.5	251.0 ± 194.8	269.4 ± 173.7	439.9 ± 297.0	96 days on Dimilin 121 days of age

[a] = 15 animals each
[b] = 8 animals each
*Significantly different from control P<.05 (one-way analysis of variance)

differences; there was no statistically significant difference between the testosterone values reported by the two clinical laboratories.

DISCUSSION

The data presented in this report indicate that Dimilin had no adverse effects on body weight or organ weights of weanling rats; however, a decrease in levels of testosterone in plasma was noted. Testosterone values were

significantly lower in the prepubertal period, but this effect of Dimilin began to disappear with the onset of puberty. Histological examination of the testicular tissues of animals having lower levels of testosterone in plasma did not reveal any Dimilin-induced changes in interstitial or germinal cells. Similarly, no changes were observed in the weights of seminal vesicles or prostates of these animals.

In conclusion, the results of this study show that Dimilin given to male weanling rats at dosage levels of up to 300 milligrams per kilogram per day transiently depresses the testosterone levels in plasma during the prepubertal period, yet has no delaying effect on the development of the reproductive organs.

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16. ABSTRACT

The effects of the subacute doses of Dimilin [1(4-chlorophenyl)-3-(2,6-difluoropbenzyl)urea] on the reproductive system of weanling male rats were examined over a period of 96 days. The parameters evaluated were: plasma testosterone level, growth of reproductive organs (testes, prostate, seminal vesicles) and adrenal glands, and histological examination of tissues for pathological changes associated with the administration of Dimilin. The animals, 25 days old, were given 0, 15, 150, and 300 milligrams/kilogram/day of Dimilin suspension in vegetable oil by intragastric intubation for a period of 0, 14, 28, 42, and 96 days. The data indicate that Dimilin had no adverse effects on body weight or organ weights of weanling rats, but a decrease in circulating testosterone in the plasma of animals of prepubertal age was noted. However, this effect of Dimilin began to disappear with the onset of puberty. The histological examination of the test animals with lower circulating testosterone in plasma failed to reveal any Dimilin-induced changes in interstitial or germinal cells. On the basis of these observations, it is concluded that Dimilin, at 15, 150, and 300 mg/kg/day dosage levels, transiently depresses the testosterone in plasma in the prepubertal period, yet has no delaying effects on the development of the reproductive organs.

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